

## PRIMER NOTE

# Sequence variation of intergenic mitochondrial DNA spacers (mtDNA-IGS) of *Phytophthora infestans* (Oomycetes) and related species

R. A. M. WATTIER,\* L. L. GATHERCOLE,\* S. J. ASSINDER,\* C. J. GLIDDON,\* K. L. DEAHL,†  
D. S. SHAW\* and D. I. MILLS‡

\*School of Biological Sciences, University of Wales, Bangor, Gwynedd, LL57 2UW, UK, †Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331–2902, USA, ‡Vegetable Laboratory, ARS, USDA, Beltsville, MD, USA

## Abstract

The potato late-blight disease is caused by the pseudofungus *Phytophthora infestans* (Oomycetes). This pathogen was of historical importance as it caused the Irish Potato Famine. There is currently a worldwide resurgence of the disease. Following worldwide migrations as well as being able to discriminate *P. infestans* from related species are key issues. We present sequence variation of five inter-genic mitochondrial DNA spacers (mtDNA-IGS) for *P. infestans* and four related taxa. Intra and inter-taxon variation was observed showing potential for both molecular ecology and molecular systematic.

**Keywords:** genetic marker, mtDNA intergenic spacers (mtDNA-IGS), *Phytophthora infestans*, potato late-blight disease

Received 22 July 2002; revision accepted 20 November 2002

The filamentous pseudofungus *Phytophthora infestans* (Oomycetes) causes late-blight disease of potato and was responsible for the Irish famine in the 1840s. The pathogen remains highly destructive, and the last 25 years have seen a worldwide resurgence in the disease which has been associated with migration(s) of the pathogen from Mexico, its centre of diversity (Fry and Goodwin 1997). In addition, the taxonomic status of many isolates from South America remains obscure (Ordóñez *et al.* 2000). In Mexico, two related taxa, *P. mirabilis* (Goodwin *et al.* 1999) and *P. ipomoeae* (Flier 2001) were only very recently recognised as distinct species.

The aim of this study was to ascertain whether noncoding intergenic mitochondrial (mt)DNA spacers (mtDNA-IGS) showed sequence variation that could (i) provide additional polymorphism within *P. infestans* to aid in population genetics and phylogeography, because so far only four mitochondrial haplotypes have been described worldwide (e.g. Griffith & Shaw 1998), and (ii) be used for molecular identification and phylogeny of species closely related to *P. infestans*.

Four primer pairs (D88, F149, H139 and P191, Table 1) were designed, and one primer pair (ATP9) was previously described in *P. cinnamomi* (Dobowolski *et al.* 1998;

Table 1) but had never been used for sequences analysis. Primers were designed from adjacent coding sequences to amplify five mtDNA-IGS and are based on the complete mtDNA genome sequence (NCBI NC\_002387) of *P. infestans*.

Of 23 isolates selected for analysis (Table 2), 15 of *P. infestans* were from UK, USA, Canada, Mexico and Ecuador, including all four mtDNA haplotypes (Ia, Ib, IIa and IIb) already described. Two isolates from *Solanum brevifolium* in Ecuador were of ambiguous taxonomic status, possibly *P. infestans* (Ordóñez *et al.* 2000). Two isolates each of *P. ipomoeae*, *P. mirabilis* and *P. phaseoli* were from Mexico.

Polymerase chain reactions (PCR) were carried out in a volume of 10 µL that included approximately 5 ng template DNA, 100 µM each dNTP, 200 nM each primer, 10 mM Tris-HCl, 50 mM KCl, 0.2 mg/mL gelatin and 0.5 Unit Taq DNA polymerase (all chemicals from Invotrogen). The concentration of MgCl<sub>2</sub> varied as described in Table 1. DNA was amplified in a Mastercycle Eppendorf thermocycler beginning with an initial denaturation at 94 °C for 2 min, followed by 35 cycles consisting of 0.5 min denaturation at 94 °C, 0.5 min annealing at a temperature indicated in Table 1, 1 min of elongation at 72 °C, and a final extension step at 72 °C for 3 min. PCR products were monitored by electrophoresis in 2.5% agarose gels. The PCR products were purified using the exonuclease and shrimp alkaline



both D88 and F149 but they were in linkage disequilibrium with the polymorphisms already described (Griffith & Shaw 1998); isolates of haplotype Ia and Ib have one sequence type whereas isolates with IIa or IIb have another. Although the combination of polymorphisms detected for ATP9 and H139 did not allow subdivision of haplotypes Ib and IIa into new haplotypes, they did allow the splitting of both Ia and IIb into three new haplotypes.

The data show that the two isolates with ambiguous taxonomic status (Pi?-ECbr1 and Pi?-ECbr2) are different from the isolates of *P. infestans* from potato (Table 2). The two Pi? isolates have identical nucleotide sequences but are polymorphic for 45 nucleotides within the five targets with respect to the reference sequence (including a 10 nucleotide deletion within H139). These two isolates are also highly polymorphic with respect to the three other species examined in this study.

The high level of variation observed among species revealed the potential of these markers in taxonomy and phylogeny. Finally, intraspecific polymorphism is observed for *P. mirabilis* at ATP9 and for *P. ipomoeae* and *P. mirabilis* at H139.

### Acknowledgements

The authors thank G. A. Forbes (CIP, Ecuador) who provided isolates Pi?-ECbr1 and Pi?-ECbr2, W. G. Flier, N. J. Grunwald and L.

P. Kroon (PRI, the Netherlands) who provided isolates of *P. ipomoeae*, *P. mirabilis* and *P. phaseoli*, and A. Stenson (University of Wales, Bangor), N. Adair and D. Borngasser (Oregon State University) for assistance in DNA sequencing. This project was funded in part by BBSRC, the USDA Agricultural Research Service, and the OSU Agricultural Experiment Station.

### References

- Dobrowolski MP, Tommerup IC, O'Brien PA (1998) Microsatellites in the mitochondrial genome of *Phytophthora cinnamomi* failed to provide highly polymorphic markers for population genetics. *FEMS Microbiology Letters*, **163**, 243–248.
- Flier WG (2001) Variation in *Phytophthora infestans*: sources and implications. PhD Thesis. Wageningen University, The Netherlands.
- Fry WE, Goodwin SB (1997) Resurgence of the Irish potato famine fungus. *Bioscience*, **47**, 363–371.
- Goodwin SB, Legard DE, Smart CD, Levy M, Fry WE (1999) Gene flow analysis of molecular markers confirms that *Phytophthora mirabilis* and *P. infestans* are separate species. *Mycologia*, **91**, 796–810.
- Griffith GW, Shaw DS (1998) Polymorphisms in *Phytophthora infestans*: four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Applied and Environmental Microbiology*, **64**, 4007–4014.
- Ordóñez ME, Hohl HR, Velasco JA *et al.* (2000) A novel population of *Phytophthora*, similar to *P. infestans*, attacks wild *Solanum* species in Ecuador. *Phytopathology*, **90**, 197–202.